

PTO 02-2101

German Patent No. 39 18 412 A1

SCANNING MICROSCOPE FOR TRANSMITTED AND REFLECTED LIGHT

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UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. APRIL 2002
TRANSLATED BY THE RALPH MCELROY TRANSLATION COMPANY

FEDERAL REPUBLIC OF GERMANY
GERMAN PATENT OFFICE
PATENT NO. 39 18 412 A1
(Offenlegungsschrift)

Int. Cl. ⁵ :	G 02 B 21/00 G 02 B 21/18 G 02 B 27/10 G 02 B 27/28
Filing No.:	P 39 18 412.9
Filing Date:	June 6, 1989
Date Laid Open to Public Inspection:	February 8, 1990
Priority	
Date:	August 3, 1988
Country:	DD
No.:	WP G 02 B/318645

SCANNING MICROSCOPE FOR TRANSMITTED AND REFLECTED LIGHT

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The invention pertains to a scanning microscope for transmitted and reflected light, which is equipped with a laser light source and which can be used particularly for examining extremely small structures and low-contrast objects in all branches of microscopy. In particular, these branches include genetic engineering, immunology, semiconductor research and technology, and other fields.

Confocal raster microscope arrangements that are equally well suited to reflected and transmitted light examinations must constantly satisfy the strict requirement that a first laser focus and a so-called coherent receiver must remain centered and fixed relative to each other at fractions of the resolving power of the microscope over long periods of time.

For purely reflected light operation, such microscope arrangements can be realized by taking advantage of the reversibility of the light path, wherein the light reflected at the object returns over the same path traversed by the illuminating light up to shortly before the coherent

receiver. This method compensates for all instabilities of the beam path and the return light is always incident on the center of the coherent receiver (DE-PS 30 37 983 and EP-PS 01 67 410).

Confocal scanning microscopes using transmitted light have not become popular before now because of their technical complexity. Such an arrangement is described in EP-PS 01 68 983 and operates with so-called object scanning. The strict requirements on the dynamic range of the object scanner only permit examination within a relatively narrow dimensional range, so that such devices cannot be used universally.

A microscope arrangement is proposed, for which object scanning with transmitted and reflected light can be performed while using one and the same scanner. However, a very complicated scanner that operates in two axes is required. Here, the imaging beam path traverses a different path than the illuminating light over the entire length (up to the objective for reflected light). This further results in strict requirements on the stability of the entire construction.

The goal of the invention is to eliminate the disadvantages of the state of the art and to simplify the construction for scanning microscopes using transmitted and reflected light and to increase the service value of these microscopes.

The task of the invention is based on creating a scanning microscope for transmitted and reflected light, for which, independently of the illumination type, the same standard, in principle, known, scanner arrangement can be used and for which, the imaging beam path for both illumination types passes through a maximum number of optical components on the same path as the illuminating light but in opposite directions.

According to the invention, the objective is realized by a scanning microscope for transmitted and reflected light with a laser as a light source, whose radiation is polarized in a predetermined azimuth, with an aperture arranged before a photoelectronic receiver and confocal to the object plane, with a beam-scanning device and two objectives that are symmetrical to the object plane and that are centered relative to the optical axis, as well as with deflection elements arranged in the beam path for illuminating the object and in the return beam path, such that,

as viewed in the direction of the light, there is a first polarization optical beam splitter before the beam-scanning device and a second polarization optical beam splitter after the beam-scanning device, and there is a $9/4$ plate behind each of the two light output surfaces of the second beam splitter,

in the light paths generated by the second beam splitter, there are deflection elements that only change the polarization state of the beam insignificantly such that the two beams passing through the second beam splitter are guided over the same optical path but in opposite directions through the objectives,

and the second polarization optical beam splitter can be switched into and out of the beam path.

With this device, which uses a small number of optical components and is independent of the illumination type, the imaging beam path travels through a maximum number of components on the same path and in the opposite direction as the illuminating light beam in the illuminating beam path. This device further offers the advantage that the illuminating light and the light changed by the object can be distinguished by the azimuth of the polarization plane and can be separated from each other shortly before the aperture arranged confocal to the object plane by the first beam splitter, wherein significant parts of the beam path are traversed in common by illuminating and changed light, so that instabilities in this part of the device do not have negative effects. In this way, the requirements for stability and adjustments to the device are also reduced to a minimum.

The invention is to be explained in more detail in the following relative to an embodiment. Shown in the drawing are:

Figure 1, schematically the beam path of the device, and

Figure 2, another variant of the beam guidance after the beam-scanning device.

As illustrated in Figure 1, a laser beam that is polarized in a predetermined azimuth and that is output from a laser 1 serving as the light source is focused by a lens 2 in the plane 3. Viewed in the direction of the light, there is a first polarization optical beam splitter 5 before a downstream beam-scanning device 4 and a second polarization optical beam splitter 6 after the beam-scanning device 4 in the beam path of the scanning microscope, wherein the first beam splitter 5 is oriented so that the polarization azimuth of the transmitted beam or beam bundle agrees with the laser 1 and consequently, the light can pass through the beam splitter 5 unhindered. A lens 7 images the plane 3 again at infinity. The lenses 2 and 7 further widen the beam leaving the laser 1 to the required diameter. In the beam-scanning device 4, the beam is then deflected towards the image point it is to intersect in the object plane 7 [sic; 18] of the two objectives 8 and 9 by the required angle with known means such as swinging mirrors, plane-parallel plates, or similar elements. A tube line [sic; lens] 10 images the laser focus produced in the beam-scanning device 4 at infinity.

For the illumination of an object lying in the object plane 18 with transmitted light, the illuminating beam passes with almost no loss through the second beam splitter 6, whose transmission azimuth coincides with the polarization azimuth of the illuminating beam, and it is guided through a deflection element 11 into the objective 8 and is focused in the object plane 7 [sic; 18]. By the objective 9, the laser focus focused in the object plane 18 is again imaged at infinity. The two objectives 8 and 9 are symmetrical to each other relative to the object plane 18 and centered in the beam path relative to the optical axis 12. By means of a deflection element 13 arranged after the objective 9, the beam is again guided into the beam splitter 6, and behind each of the two output surfaces 14 and 15 of the beam splitter there is a $9/4$ plate 16 and 17. Through

the use of two $9/4$ plates 16 and 17 or a single $9/2$ plate (not illustrated in Figures 1 and 2) arranged at the site of the $9/4$ plate 17, the polarization azimuth of the incident beam is rotated by 90° in a known way, so that the beam input into the second beam splitter 6 oscillates in the azimuth, in which it is optimally reflected. By means of the tube line 10, the beam-scanning device 4, and the lens 7, the light rotated in the polarization plane by 90° relative to the illuminating beam is guided into the first beam splitter 5 and there it is deflected optimally in the direction of a point aperture 19 that is conjugate relative to the plane 3. A local laser focus is formed in the plane of this point aperture 19, which is advantageously arranged directly before a photoelectric receiver 20, wherein the focused light holds information from the object point passed through in the object plane 18. The receiver 20 generates electronic signals that can give information about the object. If the objectives 8 and 9 are centered relative to each other and focused relative to the object plane 18, and the deflection elements 11 and 13 are tilted symmetrically to the object plane 18, then the light changed by the object passes through the components 4; 5; 6; 7 and 10 on the same optical path as the light of the illuminating beam.

For the illumination of the object arranged in the object plane 18 with reflected light, the second beam splitter 6 that is arranged in the direction of the light behind the beam-scanning device 4 is taken out of the beam path, so that the beam leaving the device and passing through the tube lens 10 and the $9/4$ plate 16 is focused by the deflection element 11 through the objective 8 in the object plane 18. The beam reflected from the object passes through the objective 8 again but in the opposite direction, is reflected at the deflection element 11, and once again passes through the $9/4$ plate 16. In this way, as in the case described for illumination with transmitted light, its plane of polarization is rotated by 90° , and the return beam can be reflected at the polarization optical first beam splitter 5 optimally in the direction of the point aperture 19 and the receiver 20.

Figure 2 shows the beam path of a scanning microscope after the beam-scanning device 4 and the tube lens 10, wherein, in the direction of the light, a polarization optical beam splitter 21 and a 90° deflection prism 22 follow in the beam path. By means of deflection elements 23 and 24 and a $9/4$ plate 27, which rotate the light by 90° , with transmitted light the beam is focused through the objective 25 in the object plane 18. The beam changed by the object is guided by means of the objective 26, the deflection elements 28 and 29, and the $9/4$ plate 30 the deflection prism 21 and there it is transmitted through the beam splitter 21 and the other components already mentioned in the description of Figure 1 to the photoelectric receiver 20. Also for this arrangement, the objectives 25 and 26 are arranged symmetrical to the object plane 18 and centered relative to each other.

For the illumination with reflected light, the beam splitter 21 is removed from the beam path. The light then passes through the deflection prism 22 and the $9/4$ plate 30, is reflected at the

deflection elements 28 and 29 to the objective 26, and intersects the object plane 18 with the object. The light changed by the object traverses the same path but in the opposite direction up to the photoelectric receiver 20 (not illustrated in Figure 2).

Claim

Scanning microscope for transmitted and reflected light with a laser as a light source, whose radiation is polarized in a predetermined azimuth, with an aperture arranged before a photoelectric receiver and confocal to the object plane, with a beam-scanning device, and two objectives that are arranged symmetrical to the object plane and that are centered relative to the optical axis and to each other, as well as with deflection elements arranged in the beam path for illuminating the object and in the return beam path, characterized in that, as viewed in the direction of the light, there is a first polarization optical beam splitter (5) before the beam-scanning device (4) and a second polarization optical beam splitter (6) after the beam-scanning device (4) and there is a $9/4$ plate (16; 17) after each of the two beam output surfaces (14; 15) of the second beam splitter (6), in the light paths generated by the second beam splitter (6) there are deflection elements (11; 13) that only change the polarization state of the beam insignificantly such that the two beams passing through the second beam splitter (6) are guided over the same optical path but in opposite directions through the objectives (8; 9), and the second polarization optical beam splitter (6) can be switched into and out of the beam path.

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